IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant: Samy Ashkar and Jairo Salcedo

Serial No.: 09/981,845 Art Unit: 1647

Filed: October 18, 2001 Examiner: Regina M. Deberry

For: *OSTEOPONTIN-COATED SURFACES AND METHODS OF USE*

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY BRIEF TO EXAMINER'S ANSWER

Sir:

This is a reply brief to the Examiner's Answer mailed September 1, 2006, and the Supplemental Examiner's Answer mailed October 17, 2006, in the above-referenced application

It is believed that no fee is required with this submission. However, should a fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-3129.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issue presented on appeal is whether claims 1-3, 5 and 6 are enabled under 35 U.S.C. § 112, first paragraph.

(7) ARGUMENT

Appellants affirm all of the arguments made in the Appeal Brief.

(a) The Invention

Osseointegration is a complex process that involves proliferation, migration, attachment, differentiation, extracellular matrix synthesis, and, finally, mineralization of that matrix. Differentiated cells originate from "primitive" cells called stem cells, which are pluripotent and divide to generate committed precursor cells. After a series of rapid cell divisions, these committed precursor cells develop into differentiated cells, wherein a contribution is made to the surrounding matrix. Driving this process of cellular development is motility and proliferation, which are in turn, regulated by increasing or decreasing gradients of, for example, peptides which bind to receptors on the cell surface. The bone trauma generated by implant placement is followed by clot formation, acute inflammation, recruitment and proliferation of stromal cells and their differentiation into osteogenic lineage cells, followed by filling of the defect with bone and, finally, mineralization of the matrix.

Extracellular matrix proteins, especially adhesion molecules, play a role in bone repair and morphogenesis. When cells initially encounter a bio-matrix or extracellular matrix ("ECM"), they will either attach and spread or undergo apoptosis. Adherence to the ECM is a receptor-mediated process. Cell surface receptors belonging to the integrin super family are recognized as critical players in the adhesion to the ECM and are intermediate messengers relaying signals for events such as contact, anchorage, and differentiation. Proteins such as osteopontin or peptides derived from osteopontin, which bind to these receptors, can therefore mediate these cellular processes.

The primary challenge faced in the fabrication of new implants is to increase the rate of osseointegration and the percentage of bone apposition. An enhanced rate of osseointegration and/or augmented percentage of bone apposition around implants increases implant placement indications, and expedites loading time. Recent studies have focused on improving osseointegration of implants by coating the surface with various substances including bone morphological proteins, with varying degrees of success.

The Appellants have isolated active osteopontin peptide fragments that have (1) cell-attachment; and (2) cell-spread activity, which, when coated on a material suitable for use as an implant, can increase cell attachment as well as enhance cell spread. The peptides discovered by the Appellants help bring stem cells, precursor cells, and differentiated cells into contact with the material. They can also function in bringing tissue remodeling cells such as mesenchymal, macrophages and granulocytes and, in general cells that are involved in osseointegration, into contact with the implant.

(b) Rejection of claims 1-3, 5 and 6 Under 35 U.S.C. § 112, first paragraph, enablement

The Legal Standard for Enablement

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. See, e.g., *Amgen v. Hoechst Marion Roussell* 314 F.3d 1313 (Fed. Cir. 2003) and *Genentech, Inc. v. Novo Nordisk A/S*, 108 F3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v. Telectronics, Inc.*, 857 F.2d 778

(Fed. Cir. 1988); and *In re Stephens*, 529 F.2d 1343 (CCPA 1976). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985). The adequacy of a specification's description is not necessarily defeated by the need for some experimentation to determine the properties of a claimed product. *See Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F3d 956, 965-966 63 USPQ2d 1609, 1614 (Fed. Cir. 2002). In addition, a patent need not teach, and preferably omits, what is well known in the art. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *citing Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 U.S.P.Q. 481, 489 (Fed. Cir. 1984). Thus, information that is conventional or well-known to one of ordinary skill in the art need not be disclosed by the specification.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir.1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors involved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation 'must not be unduly extensive.' *Atlas Powder Co., v. E.I. DuPont De Nemours*

& Co. , 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir.1984). There is no requirement for examples. The Supreme Court also noted that all of the factors need not be reviewed when determining whether a disclosure is enabling *In re* Amgen, Inc. v. Chugai Pharm. Co., 927 F.2d 1200, 1213, 18 USPQ2d 1016, 1027 (Fed. Cir. 1991) (noting that the *Wands* factors 'are illustrative, not mandatory. What is relevant depends on the facts.'). As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied. *In re Douglas v. United States* 510 F.2d 364; 184 U.S.P.Q. 613 (Ct. Cl.1975) the Court of Claims noted that a patentee cannot "be expected to foresee every technological problem that may be encountered in adapting his idea to a particular use. Some experimentation and exercise of judgment is to be expected. "Enablement is not precluded by the necessity for some experimentation such as routine screening." *In re Wands*, citing to *Minerals Separation, Ltd. v. Hyde*, 242 U.S. 261, 270-71 (1916), wherein the court emphasized that some inventions cannot be practiced without adjustments being made to adapt them to the particular context. In such a situation, a specification is sufficient if it gives adequate guidance to one skilled in the art on how such adjustments are to be made.

Analysis

Claims 1 ,2, 3, and 5

Claim 1 defines an active osteopontin peptide fragment comprising an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein the peptide binds to at least one integrin receptor on a cell surface selected from the group consisting of αvβ3, αvβ5, 4β 1, 2β1, VCAM, ICAM CD44, V3Vx.

An active osteopontin peptide refers to an osteopontin fragment that possesses at least one biological activity of naturally occurring osteopontin (see page 11, lines 9-11). The biological activity of osteopontin that the claimed peptides have includes cell attachment and cell spreading activity. Osteopontin performs these biological functions by binding to receptors on the cell surface. It is well known in the art that osteopontin binds to more that one integrin receptor, as is exemplified by Hu, et al, *J. Biol. Chem.* 270(11):26232-38 (1995) ("Hu") (Submitted in the Appeal Brief Evidence). The blast 2 sequence comparison (submitted with Appeal Brief, a copy of which is attached) shows, for example, that SEQ ID NO 11 and 15 have conserved domains similar to osteopontin. The ability of the peptides recited in claim 1 to bind to ***at least one*** integrin receptor on the cell surface is demonstrated by the ability of anti-integrin antibodies to inhibit cell attachment (for example, SEQ ID NO: 15, (Table 8)). This example clearly demonstrates that the claimed peptides do indeed bind to integrins.

The specific amino acid sequences of the peptides are disclosed at page 8, lines 7-26. The peptides can be made from osteopontin or using recombinant or synthetic techniques. The specification on page 11, lines 9-11 and on page 12, lines 20-31 to page 13, lines 1-5, discloses how osteopontin can be modified to obtain the claimed peptides. There is no legal requirement that the claimed peptides bind all integrins or all cell types for the peptides to have the specified utility. There is no legal requirement for actual reduction to practice. As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied.

Since the sequences which are responsible for binding as claimed are described, no undue experimentation would be required. One skilled in the art would simply make peptides including

the claimed sequence, and then verify the binding properties using standard, routine techniques as described in the application.

According to the MPEP §2164, "The enablement requirement refers to the requirement of 35 U.S.C. 112, first paragraph that the specification describe how to make and how to use the invention. **The invention that one skilled in the art must be enabled to make and use is that defined by the claim(s) of the particular application or patent**". The issue should therefore be whether the specification enables one of ordinary skill in the art to make the active osteopontin fragments comprising the amino acid sequence selected from the group of sequences listed in claim 1, wherein the peptide binds to at least one integrin receptor on a cell surface selected from the group of receptors listed in claim 1. Therefore claim 1, which recites that peptides consisting of SEQ ID Nos. 7-15, bind to at least one integrin receptor on a cell surface selected from the group of receptors recited in claim 1, claim 3 which recites that the peptide fragments of claim 1 bind to ***at least one*** integrin receptor selected from the integrin receptors listed in claim 3, and claim 5, which recites that the peptide fragments of claim 1 bind to at least on integrin on a cell surface, wherein the integrin is selected from the group consisting of the integrins listed in claim 5, are enabled.

The peptides may be used to increase cell attachment to a biomaterial and to increase cell spreading. The specification on page 13, line 14 to page 14, line 2 describes how to coat the peptides on a material, and the types of materials that may be coated (page 10, lines 16-23 and page 14, lines 22-28). The specification on page 40, lines 4-31, and page 41, lines 1-8, describes how to measure cell attachment and cell spreading. Example 12 shows that the claimed peptides increase cell attachment and cell spreading.

The Examiner alleges that the instant invention is drawn to osteopontin peptide fragments which induce osseointegration on implants into surrounding tissue, and that the biological function of the claimed peptides is osseointegration, not just attaching and spreading (Emphasis added by the Examiner). Appellants again respectfully draw the Examiner's attention to MPEP §2164, which states that "The enablement requirement refers to the requirement of 35 U.S.C. 112, first paragraph that the specification describe how to make and how to use the invention. The invention that one skilled in the art must be enabled to make and use is that defined by the claim(s) of the particular application or patent". Claim 2 defines an active osteopontin peptide fragment comprising the amino acid sequences selected from the group consisting of the sequences listed in claim 1, which bind to at least one integrin receptor selected from the group listed in clam 1, and wherein the peptide increases cell attachment and cell spread. As already discussed above, the specification is enabled to make the claimed peptides which bind to at least one integrin receptor and increases cell attachment and cell spread. Furthermore, with respect to the Examiner's assertion about the biological function of the peptides, Appellants respectfully draw the Examiner's attention to the specification at least at page 1, lines 9-14, wherein the physiological processes that lead to successful osseointegration are discussed. The claims define peptides which have effects on some of the physiological processes that are involved in osseointegration. A definition of osseointegration as proposed by the Examiner in the Examiner's Answer on page 11, with added emphasis, suggests that a peptide such as that defined by the claims, that increases cell attachment and spreading would not induce osseointegration of implants. This is actually not the case. The defined peptides would be useful in increasing osseointegration by virtue of the fact that they can increase cell attachment and spreading which are biological events involved in osseointegration (please see a review of

osseointegration in O'Neal, et al., *J. Oral Implant.*, XVIII(3):243-255, 1992, submitted with the Information Disclosure Statement filed on September 23, 2002, "O'Neal", a copy of which is attached in an Appendix to this Reply Brief). According to O'Neal, "with regard to interactions between implants and tissue, important events include platelet aggregation and wound healing. Concurrently, appropriate cells must migrate and attach to the implant site for establishment of an implant-tissue interface that is biocompatible" (please see O'Neal, page 244). Therefore, not only is claim 2, which recites osteopontin peptide fragments which bind to at least one integrin receptor of the surface of a cell and increases attachment to a material and increases cell spreading, enabled, the claimed peptides would also be useful for the biological process of osseointegration.

Claim 6

Claim 6 defines an active osteopontin peptide fragment comprising an amino acid sequence selected from the group of sequences listed in claim 1, wherein the peptide binds to at least one integrin receptor on a cell surface selected from the group listed in claim 1, and wherein the cell is selected from the group of cells listed in claim 6. The proper analysis for enablement of claim 6, is whether the specification enables a skilled artisan to make the peptides listed in claim 1, which can bind at least one integrin receptor on a cell surface, wherein the cell is selected from the cells listed in claim 6. The specification clearly enables a skilled artisan to make and use the peptides as defined by the claims.

The specification describes a number of cell types that may be regulated using the active osteopontin peptides fragments (page 8, line 29 to page 9, line 2). Example 12 and Table 8 on pages 53-55, demonstrate that plates coated with each of, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14 or SEQ ID NO:15, bind to one of

those cell types (osteoprogenitor cells). Table 8 shows that SEQ ID NO: 15, to be due to binding to the αvβ3 receptor. Claim 6 does not require that the peptide bind all the integrin receptors listed in claim 1. The claim requires that the peptide bind at least one integrin receptor. Appellants have already demonstrated using nothing move than routine methods, the ability to determine that an active osteopontin peptide fragment (SEQ ID NO: 15) binds to at least one integrin receptor on the cell surface of osteoprogenitor cells.

Integrins are the principal receptors on animal cells for binding most extracellular matrix proteins, including collagen, fibronectin, and laminin. They are found on the surface of numerous cell types, as demonstrated by textbooks such as *Molecular Biology of the Cell.* IV. Cells in Their Social Context. 19. Cell Junctions, Cell Adhesion, and the Extracellular Matrix, Garland Publishing (1994). This fact is also exemplified in Hu and Tuck, et al, *J. Cell. Biochem.*, 78:465-75 (2000) ("Tuck") (submitted in the Appeal Brief Evidence Appendix). Osteopontin will bind to different cell types that express its receptors. The cells employed by Hu are carcinoma cells, whereas Tuck employs epithelial cells. These references demonstrate that osteopontin will bind to at least one of its receptors on a cell expressing the receptor. The claims define active osteopontin peptide fragments. The specification at least at page 11, lines 9-11 defines "an active osteopontin peptide" as an osteopontin fragment that possesses at least one biological activity of a naturally occurring osteopontin, such as chemotactic or cell attachment activity. The peptides listed in claim 1 are modeled after osteopontin. Osteopontin performs its biological functions by binding to its receptors on cell surface. Appellants showed in Table 8 that one such peptide (SEQ ID NO: 15) can bind integrin receptors on osteoprogenitor cells. There is no requirement that every receptor be tested. Furthermore, the claim requires that the peptide bind at least one receptor. Appellants submit that the specification is clearly enabled.

The Examiner alleges that the specification only teaches that bone cells (osteoprogenitor cells) have osseointegration activity and that the specification and the art of record fail to teach that the other cell types recited in claim 6 have osseointegration activity. It is not clear to Appellants how this applies to the issue of enablement for claim 6, i.e. whether a skilled artisan can make the peptides listed in claim 1, that can bind to at least one integrin receptor on the surface of cells selected from the list of cells listed in claim 6. Furthermore, Appellants are not exactly sure what the Examiner means by a cell having osseointegration activity. As is disclosed in the specification at least at page 29, lines 3-12, the interaction of macromolecules (cytokines and adhesion molecules, that orchestrate the course of wound healing and osteogenesis) with the implant surface determines to a measurable extent how well the implant is integrated. It is clear from the discussion in the specification and in O'Neal, that successful osseointegration requires both wound healing and osteogenesis. Please see Table 1 in O'Neal, for a listing of cells (macrophages, endothelial cells, epithelial cells, hematopoietic cells, listed in claim 6) that secrete cytokines necessary for wound healing or bone remodeling, necessary for osseointegration. The specification at least from page 3, line 8, until page 4, line 14, describes the relevance of stem cells, precursor cells and differentiated cells in ossoeintegration. The specification also discloses tissue remodeling cells at least at page 15, lines 10-20. Clearly, all the cells listed in claim 5 are relevant as far as osseointegration is concerned.

(c) Summary and Conclusion

Appellants maintain that the Examiner has provided only argument in support of her allegation that the claims are not enabled. The invention that one skilled in the art must be enabled to make and use is that defined by the claim(s) of the particular application or patent. There is sufficient disclosure in the specification, and coupled with knowledge in the art, a

skilled artisan would be able to make the peptides defined by the claims which bind at least one integrin receptor on a cell selected from the cells listed in claim 6, and increase cell attachment to a biomaterial and cell spread as required by claim 2.

For the foregoing reasons, Appellant submits that claims 1-3, 5 and 6 are patentable.

Respectfully submitted,

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SUPPLEMENTAL EVIDENCE APPENDIX

O'Neal

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Biological Requirements for Material Integration

Abstract

The recognition that synthetic devices can provide functional replacements for failed teeth, or for previously edentulous areas, has resulted in increased emphasis being placed on understanding of the interactions between synthetic materials and host tissues in order for the success of these devices to be optimized. A key to achievement of an optimal biological interface between the implant and the surrounding tissue is through an understanding of host response to materials. This article reviews the biological requirements for implant-tissue integration, with specific focus on the role of adhesion molecules and cytokines (growth factors) in this process. Adhesion molecule/cytokine interactions are discussed, and in particular the possible role for osteopontin, an adhesion molecule as well as a cytokine, is considered in wound healing. Finally, the causes of peri-implantitis are discussed, and methods of decontamination are presented. The decontamination methods focus on enhancement of cell adhesion and integration to the altered implant surface.

Introduction

The functional replacement of failed teeth and edentulous areas was, until recently, relegated to the design and fabrication of fixed and removable appliances that would supplant a functional or aesthetic disharmony. Many times, such treatment resulted in poor esthetics, limited function, or a lack of compliance. In order for these limitations to be overcome, a variety of synthetic devices has evolved that may be implanted and integrated within the masticatory apparatus for symmetry and function in the host to be achieved. As a result, implantation of synthetic devices has mandated an increased emphasis on understanding of the tissue-material interactions so that the success of such implants can be optimized. For example, in order for an optimal biological interface between the implant and surrounding tissue to be achieved, termed osseointegration (Brånemark, 1985), the composition of the implant must be balanced with the surgical procedures necessary for placement and the long-term stresses anticipated during its lifetime. In addition, several host factors need to be considered in order for optimal conditions for placement and function of implants to be provided (Ziats *et al.*, 1988; Donley and Gillette, 1991; Galante *et al.*, 1991; Jaffin and Berman, 1991). Patient medical and dental history, including the quality of existing soft and hard tissues of the oral cavity, must be weighed prior to the institution of therapy. Specific attention must be paid and precautions should be taken with patients in whom these sequelae were the result of rapidly progressive periodontal disease. Malestrom *et al.* (1990) indicate that in such patients, complications that mimic the primary disease might predispose to the failure of the implanted device. Consequently, patients with a significant history of periodontal disease warrant distinctive mechanical and antimicrobial considerations prior to the placement of dental implants. Moreover, research efforts have centered on the recognition of early signs of failure, *e.g.*, peri-implantitis, excessive functional stress, in order for loss of implants to be prevented (Hickey *et al.*, 1991).

An index of a successful implant is the achievement of osseointegration between the device and surrounding tissues. Osseointegration is defined by Brånemark (1985) as "direct structural and functional contact between ordered, living bone and the surface of a load-carrying implant". At the microscopic level, bone tissue is frequently found in close contact, 100-900 microns, but not in direct contact with the implant surface. Because the establishment of this zone is critical to the success of an implant, significant effort has been focused on determination of the composition, nature, and properties of this interface (Buser *et al.*, 1991). Accomplishment of an optimal interface would reduce failures that are seen and considered to be the result of or related to fracture or enhanced bone resorption and fibrous tissue formation.

The biological requirements for implant-tissue integration are discussed in this review, with specific emphasis on the possible role of cytokines (growth factors) and adhesion molecules in this process. The term "cytokine" has been used in references to polypeptides synthesized by or acting on hematological cells for the regulation of cell growth and differentiation, whereas the term "growth factor" has been used for molecules having similar activities, but acting and/or synthesized by other cell types (Canalis *et al.*, 1991; Albelda and Buck, 1990). These terms are used interchangeably here. In addition, the potential for a failing implant interface to be restored is presented in terms of provision of a surface that will allow for re-attachment of cells at the implant-tissue interface.

Tissue Healing at the Implant Site

In order for cells to interact appropriately with their environment, they must be able to adhere to supporting substrata. Such interactions are important to many physiological and pathological events, which include platelet aggregation (Dixit *et al.*, 1985; Ginsberg *et al.*, 1985), wound healing (Grinnell, 1984), embryogenesis (Boucaut *et al.*, 1984; Thiery, 1984), and malignant invasion and metastasis (Vaheri and Mosher, 1978; Ruoslahti, 1984; Humphries *et al.*, 1986). These events are modulated through a variety of cellular activities which include cell-cell communication, cell-substratum anchorage-dependent interactions, proliferation, migration, differentiation, and matrix synthesis. With regard to interactions between implants and tissues, important events include platelet aggregation and wound healing. Concurrently, appropriate cells must migrate and attach to the implant site for establishment of an implant-tissue interface that is biocompatible. Specific factors are involved in the regulation of cells in the local area and include cytokines and adhesion molecules. The interaction of cells with a substratum, whether it is an implant material or a biological extracellular matrix (ECM), will trigger cells to synthesize specific cytokines (Nathan and Sporn, 1991). These cytokines have been shown, subsequently, to regulate the synthesis of specific adhesion molecules, which in turn will control future cell-cell and cell-substratum (implant) interactions.

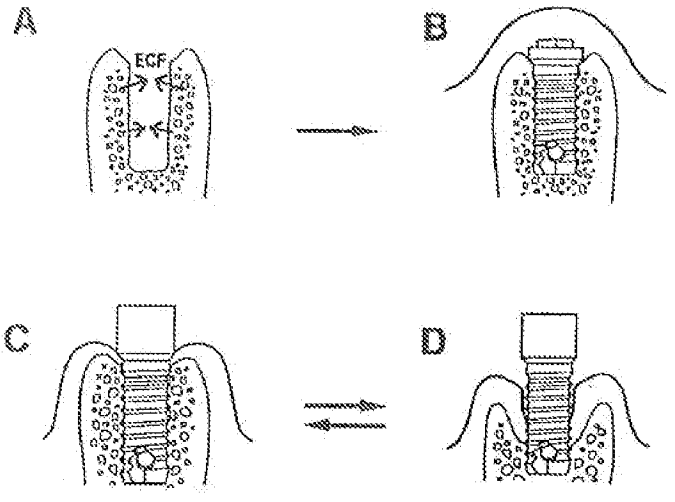

Figure 1. Implant-host interactions. (A) Implant-prepared site demonstrating potential interactions of extracellular fluid (ECF) and cells from surrounding tissues. (B) Insertion of implant and interactions of implant with cells/factors in the local environment. (C) Stable host-implant environment. (D) Peri-implantitis.

Initial events at the wound-healing site include introduction of the implant to the extracellular fluids (ECF) and cells at the prepared (wound) site (Fig. 1, b). It is now recognized that cytokines and cell adhesion molecules play a critical role in determining which cells will populate a given area and the ability of these cells to react with other cell types, with the extracellular matrix, and with the implantable materials in the local environment. Conversely, the local environment and the implant material can alter the responses of cells to local factors. An additional complexity is that, during the life of the artificial device, proteins in the local environment may change; that is, certain factors may be absorbed from the implant and replaced by other proteins at that site (Vroman, 1988; Anderson *et al.*, 1990).

Cytokines

Cytokines are produced by a variety of cell types and have diverse bioactivities which include promotion and/or inhibition of cellular proliferation and differentiation, depending on the specific cell type. The potential importance of these diverse activities has resulted in increased efforts at understanding the role of specific growth factors as they relate to individual tissues. Comprehensive reviews on cytokines as they relate to several fields—including immunology, virology, hematology, and cell biology—are available (Krane *et al.*, 1988; Wahl *et al.*, 1989; Kasperk *et al.*, 1990; Robinson and Quesenberry, 1990a,b; Nathan and Sporn, 1991; Canalis *et al.*, 1991; Mohan and Baylink, 1991; Lynch, 1991).

Table 1 presents a modified list of cytokines that may prove important for the achievement of adequate implant-tissue integration. Growth factors have been implicated as having a role in the process of wound healing. In particular, cytokines may increase the rate of wound closure, increase the rate of proliferation of fibroblasts and epithelial cells at the site of healing, increase the rate of vascularization, and enhance the rate of collagen deposition. Many cytokines concentrate in mineralized tissue and have been isolated and characterized from these tissues, as well as from media obtained from bone cells and bone tissues, *in vitro*. In addition,

TABLE 1
CYTOKINES AND IMPLANTS*

Factor	Location	Suggested Function	Reference
PDGF	Platelets Endothelial cells Macrophages Bone	Bone remodeling, wound healing	Centrella *et al.*, 1989 Lynch *et al.*, 1991a,b
IGF-I and II	Plasma Liver Bone/cartilage	Wound healing, bone formation	Czech, 1989 McCarthy *et al.*, 1989 Mohan and Baylink, 1991 Lynch *et al.*, 1991a,b
TGF-β super gene family	Platelets Bone	Wound healing, bone homeostasis, induce TIMP, immune reactions	Wozney *et al.*, 1988 Mohan and Baylink, 1991 Jones *et al.*, 1991
BMP-1	Bone	Bone induction	Wozney *et al.*, 1989
FGF a,b	Brain Bone	Endothelial cell proliferation, bone remodeling, angiogenic factors, induce MP	Globus *et al.*, 1988 Graves and Cochran, 1990 Mohan and Baylink, 1991 Canalis *et al.*, 1991
TGF-α	Macrophages Epithelial cells	Cell growth & differentiation	Massagué, 1990
EGF	Submaxillary glands Platelets	Cell differentiation, induces MP, epidermal growth & keratinization, wound healing	Edwards *et al.*, 1987 Cohen, 1983
IL's	Hematopoietic origin	Inflammatory & immune reactions, bone regulation, hematopoiesis, induce MP	Durum *et al.*, 1985 Cross and Dexter, 1991 Canalis *et al.*, 1991 Nguyen *et al.*, 1991
TNF-α,β	Hematopoietic origin	Osteoclast activation, inflammatory & immune reactions, induces MP	Canalis *et al.*, 1991 Paul and Ruddle, 1988
CSF's	Hematopoietic origin	Immune reactions, regulation of bone marrow cells	Robinson and Quesenberry, 1990a,b
INF-γ	Hematopoietic origin	Inflammatory & immune reactions	Defilippi *et al.*, 1991a,b
Eta-1 (OPN)	Mineralized tissues Activated T-lymphocytes	Host resistance, mineral homeostasis	Butler, 1989 Patarca *et al.*, 1990 Reinholt *et al.*, 1990

*Provided here are primary locations and functions of cytokines as related to implants. Cytokines have diverse activities and are associated with several tissues.

Abbreviations: TIMP, tissue inhibitor of metalloproteinases; MP, metalloproteinases; EGF, epidermal growth factor; FGF a,b, fibroblast growth factor (acidic/basic); IL's, interleukins; TNF, tumor necrosis factor; TGF-β, transforming growth factor β; CSF, colony-stimulating factor; Eta-1 (OPN), osteopontin; PDGF, platelet-derived growth factor; INF, interferon; BMP-1, bone morphogenic protein 1; IGF, insulin growth factor.

several cytokines bind to extracellular matrix molecules such as proteoglycans, which are thought to provide control over the localized concentration and action of cytokines.

Growth factors that have been associated with bone include transforming growth factor β supergene family (TGF), acid and basic fibroblast growth factors (FGF), platelet-derived growth factors (PDGF), and insulin-like growth factors (IGF I and II). Increasing evidence suggests that an important function for these cytokines, which act in concert with each other, is in the regulation of bone formation and resorption (Mohan and Baylink, 1991). The most versatile of these cytokines, the TGFβ family, is encoded with several different genes, among which are those for osteoinductive proteins [BMP's—bone morphogenic proteins, including BMP-3 (osteogenin)] (Urist, 1965; Wozney *et al.*, 1988; Katz and Reddi, 1988; Sampath *et al.*, 1990; Reddi and Cunningham, 1990; Harrison *et al.*, 1991). One of the BMP's, BMP-1, appears to be a unique regulatory molecule, not related to the TGFβ family, capable of inducing cartilage formation *in vitro* (Wozney *et al.*, 1988). The insulin-like growth factors, IGF-I (formerly called somatomedin C) and IGF-II (formerly skeletal growth factor), appear to be regulated by osteotropic hormones (Mohan and Baylink, 1991). IGF's are bound to specific binding proteins in serum and other extracellular fluids. Interestingly, one of these binding proteins, BP28 (based on molecular weight), contains an arginine-glycine-aspartic acid (RGD) cell adhesion domain (Czech, 1989). Thus, BF28-bound IGF may have a physiological role in the regulation of cell attachment. Platelet-derived growth factor (PDGF) is a dimer of two genes, PDGF A and B, with three forms existing, *i.e.*, PDGF AA, PDGF BB, and PDGF AB. Acidic and basic fibroblast growth factors (FGF a,b) are members of the heparin-binding growth factor family (HBGF) and are strong promoters of blood vessel growth, *in vivo* (Ingber and Folkman, 1989).

Other cytokines not found in high concentration in bone, but those associated with wound healing are also listed in Table 1. These include: (a) epidermal growth factor (EGF) and transforming growth factor α (TGFα), which share structural and functional homology; (b) interleukins (IL's), at least eight of which have been recognized as having diverse actions and effects on a wide range of cell types; (c) tumor necrosis factor α and β (TNFα, β) are very similar molecules, with similar activities as related to inflammation and anti-tumor activity (The primary source of TNFα is macrophages and of TNFβ is T-lymphocytes.); (d) colony-stimulating factors (CSF's) (There are four well-characterized CSF's that have a role in development of bone marrow precursor cells: [multi-CSF [IL-3], M-CSF [macrophage-CSF], G-CSF [granulocyte-CSF], and GM-CSF [granuloctye macrophage-CSF]]; (e) interferon γ (INF-γ), which modulates inflammation and immune reactions; and (f) OPN (Eta-1), an adhesion molecule first identified in bone, but now associated with several other tissues, including the identification of this molecule as a cytokine in activated T-lymphocytes, which is discussed in more detail later. [OPN (osteopontin) has been given several names, which include: (1) 44-kilodalton bone phosphoprotein (44-kDa BPP), based on molecular-weight sedimentation equilibrium (Prince and Butler, 1987; Prince *et al.*, 1987); (2) osteopontin, suggesting a bridging potential between cells and mineral bone (Oldberg *et al.*, 1986); (3) 2ar, a tumor promoter inducible protein secreted by mouse JB6 epidermal cells (Smith and Denhardt, 1987; Craig *et al.*, 1988); (4) mouse-secreted phosphoprotein I (Sppl) (Fet *et al.*, 1989); (5) tumor-secreted phosphoprotein, secreted by spontaneously transformed cells, is related if not identical to OPN (Senger and Perruzzi, 1985; Senger *et al.*, 1988); (6) BSP-I, bone sialoprotein I (Fisher *et al.*, 1987); and (7) Eta-1 (Early T-lymphocyte activation gene), based on its expression in activated T-lymphocytes (Patarca *et al.*, 1989; Singh *et al.*, 1990).]

Growth factors associated with bone have been evaluated for their potential use in the regeneration of bone tissue lost as a consequence of periodontal disease. Using beagle dogs with naturally occurring periodontal disease, Lynch *et al.* (1991b) have reported that PDGF (AB and BB) and IGF-I in combination can enhance periodontal regeneration. For these studies, they used a rapidly degradable methylcellulose gel for delivery of these factors. Our group, using an *in vitro* periodontal cell model system, has shown that growth factors and adhesion molecules, specifically FGFb and fibronectin, maintain their biological activity when incorporated into a slow-release, biodegradable polymer (Yewey *et al.*, 1992). Most recently, Lynch *et al.* (1991b) initiated studies to determine whether PDGF and IGF-I in combination would be valuable for enhancing bone formation and, subsequently, osseointegration at implant sites. While an attractive concept for improving implant sites, sufficient information is not yet available to support the value of such treatments. Nevertheless, the use of slow-release biodegradable polymer systems, which incorporate cytokines and/or other factors, may be advantageous for enhancing bone quality at implant sites.

Adhesion Molecules

Adhesion molecules associated with cytokines are reviewed, as are adhesion molecules that may be localized to an implant site. There is increasing evidence that such associations are important for signaling cells to synthesize or respond to these or other extracellular matrix molecules.

There are several types of adhesion molecules

interacting with specific ligands to promote cell attachment (Ruoslahti and Pierschbacher, 1987; Hynes, 1987; Stoolman, 1989; Hemler, 1990; Humphries, 1990; Larson and Springer, 1990; Albelda and Buck, 1990; Montefort and Holgate, 1991; Milan *et al.*, 1991; Nathan and Sporn, 1991). These include: (a) the integrin family, which are heterodimers involved in cell-substrata and cell-cell adhesion; (b) cell-surface proteoglycans; (c) cadherins, calcium-dependent adhesion; (d) selectins, cell-surface proteins with a lectin domain and an EGF-like module (including the lymphocyte homing receptors and endothelial-leukocyte adhesion molecule—ELAM); (e) the immunoglobulin superfamily, associated with embryogenesis; and (f) the 85-kDa and 67-kDa membrane proteins that bind hyaluronic acid and laminin, respectively.

Many of the adhesive molecules associated with regulation of cell response to cytokines are related to the integrin family (Table 2). The integrin complexes are a family of structurally related heterodimeric molecules, having noncovalently associated α and β subunits, that usually bind to arginine-glycine-aspartic acid (RGD) sequences present in many adhesive proteins (Hynes, 1987; Ruoslahti and Pierschbacher, 1987; Albelda and Buck, 1990; Milan *et al.*, 1991; Hemler, 1990; Larson and Springer, 1990). Initial studies suggested that there were three major subunits, β_1, β_2, and β_3, that complexed with a specific set of α subunits. It is now recognized that the integrin family is much more complex, with additional α and β subunits (Table 2).

The β_1 subfamily is composed (at a minimum) of six related complexes, each having a common β_1 chain, but a distinct α chain, each of which promotes different ligand-binding specificity. Ligands that bind to β_1 receptors include fibronectin, collagens, and laminin. The β_2 subunit, along with several related complexes, mediates primarily leukocyte cell-cell interactions that are important to normal immune responses and inflammatory cell functions (Springer, 1990; Montefort and Holgate, 1991). Ligands related to this family of receptors include endotoxin, fibrinogen, ICAM-1,2, and complement component C3bi. Another subfamily of receptors are those complexing with the β_3 subunit and include the $\alpha_v\beta_3$ receptor expressed by several cell types and having several binding ligands (Cheresh and Spiro, 1987; Smith and Cheresh, 1988; Pytela *et al.*, 1987; Sauk *et al.*, 1991; Somerman *et al.*, 1991) and the IIβ/IIIα complex expressed by platelets. The adhesion molecules associated with β_3 include thrombospondin, vitronectin, fibronectin, the von Willebrand factor, fibrinogen, OPN, and bone sialoprotein (Charo *et al.*, 1990; Albelda and Buck, 1990). The α_v subunit complexes with several β subunits, including β_1 (Vogel *et al.*, 1990), β_3 (Pytela *et al.*, 1985), β_5 (Sonnenberg *et al.*, 1988; Cheresh *et al.*, 1989; Ramaswamy and Hemler, 1990; McLean *et al.*, 1990), β_6 (Sheppard *et al.*, 1990), and β_8 (Moyle *et al.*, 1991). The α_6 subunit is associated with both β_1 (Sonnenberg *et al.*, 1991) and β_4 (Kajiji *et al.*, 1989; Suzuki and Naitoh, 1990), where a proposed ligand for $\alpha_6\beta_4$ is laminin (Hemler *et al.*, 1989). The $\alpha_7\beta_5$ integrin has been associated with the ligand vitronectin. The α_7 subunit is linked with both β_1 and β_7, where β_7 is considered, based on structural similarity with β_2, to be a member of the leukocyte cell adhesion molecule subset (Yuan *et al.*, 1991; Erle *et al.*, 1991).

TABLE 2

ADHESION MOLECULES AND CYTOKINES

Integrin	Adhesion Protein	Cytokine*
β_1, (α_1-α_6, α_v)	Laminin	TGF-β
	Collagen	TNF-α
	Fibronectin	IFN-γ
		FGFb
		IL's
β_2, (α_L, α_M, α_X)	ICAM's	CM-CSF
	Fibrinogen	TGF-β
	C3bi	TNF-α
		IL's
β_3, (α_v, α_{IIb})	Vitronectin	TGF-β
	Thrombospondin	TNF-α
	Fibrinogen	INF-α
	von Willebrand factor	Eta-1 (?)**
	OPN (Eta-1)**	
	BSP	
	FN	
β_4, α_6	Laminin (?)	ne
β_5, α_v	Vitronectin	ne
	FN	
β_6, α_v	FN	ne
β_7, α_4	VCAM	ne
β_8, α_x	ne	ne

*Cytokines associated with up- or down-regulation of adhesion molecules (receptor and/or ligand).
**OPN (Eta-1)—see text for explanation.
ne = not established; (?) = not confirmed.

Cytokines and Adhesion Molecules

There is substantial evidence indicating that cytokines affect cell adhesion molecules and that adhesion molecules can regulate the cellular expression of cytokines (Table 2). For example, TGFβ upregulates the β_1 receptor on fibroblasts (Heino *et*

al., 1989), the β_2 receptor on monocytes, and the $\alpha_v\beta_1$ receptor on osteoblasts (Ignotz *et al.*, 1989). Also, TGFβ enhances the production by osteoblasts of the bone-associated adhesion molecule OPN (Wrana *et al.*, 1991). The $\alpha_m\beta_2$ receptor on leukocytes is upregulated upon stimulation with various substances, including lipopolysaccharides and the cytokines, IL-1 and TNF. This receptor appears to be important for leukocyte adhesion to endothelial cells, a critical event for leukocyte emigration to inflammatory sites. Also, cytokines activate the VCAM-1 adhesion molecule (vascular cell adhesion molecule) on endothelial cells, and VCAM-1 binds to the $\alpha_4\beta_1$ integrin on lymphocytes. This interaction correlates with enhanced binding of lymphocytes to activated endothelial cells (Elices *et al.*, 1990). Some cytokines induce the tissue inhibitor of metalloproteinases (TIMP), *e.g.*, TGF_β and IL-6, while others, *e.g.*, FGF and EGF, are inducers of metalloproteinases (MP), where both activities alter cell adhesion (Werb *et al.*, 1989). GM-CSF and TNFα appear to play a role in decreasing expression of selectin receptors on inflammatory cells, while increasing the number of cell-surface β_2 integrins (Griffin *et al.*, 1990). Defilippi *et al.* (1991a,b) reported that TNFα and IFN_γ induced a selective down-regulation of the $\alpha_v\beta_3$ receptor on endothelial cells, while TNFα induced expression of the $\alpha_1\beta_1$ integrin on endothelial cells from the umbilical vein, a cell type which normally does not express this receptor. Using skin fibroblasts and MG-63 osteosarcoma cells, Santala and Heino (1991) demonstrated that both TNFα and IL-1 increase the biosynthesis of the α_1 integrin subunit. Moreover, TGFβ had an additive effect on IL-1's induced enhanced expression of the α_1 integrin subunit.

Conversely, several studies have shown that the extracellular matrix can regulate the cellular expression cytokines. For example, monocyte adherence to serum-coated plastic induces PDGF (Shaw *et al.*, 1990), and adherence of macrophages to fibronectin induces GM-CSF (Thorens *et al.*, 1987) and CFS-1 (Eierman *et al.*, 1989). Furthermore, the concentration of the adhesion protein can determine the response of cells to cytokines, *e.g.*, FGF will cause proliferation or differentiation of endothelial cells, depending on the concentration of fibronectin or collagen (Ingber and Folkman, 1989).

Osteopontin: An adhesion molecule and a cytokine

The awareness of the interrelationship between cytokines and adhesion molecules and the importance of these interactions to cell function have resulted in increased efforts directed at improved understanding of the signals controlling the responses of cells to such molecules. An interesting molecule that may provide some clues to cytokine-adhesion signal mechanisms is OPN. OPN, a cell adhesion protein first identified in bone, but now associated with other tissues as well, is a phosphorylated glycoprotein containing an RGD cell-binding sequence (Oldberg *et al.*, 1986; Prince *et al.*, 1987; Somerman *et al.*, 1987). In mineralized tissues, OPN is expressed prior to mineralization, is regulated by osteotropic hormones, binds to hydroxyapatite, and enhances osteoclast and osteoblast adhesion (for review, see Butler, 1989, 1991; Somerman *et al.*, 1990; Reinholt *et al.*, 1990). OPN is also associated with kidney, placenta, neurosensory cells of the ear, macrophages, smooth muscle, blood, and human milk (Nomura *et al.*, 1988; Senger *et al.*, 1989; Fet *et al.*, 1989; Butler, 1989; Patarca *et al.*, 1990; Giachelli *et al.*, 1991). In addition, OPN is expressed in many transformed fibroblasts and epithelial cells *in vitro* (Smith and Denhardt, 1987) and is found at elevated levels in plasma of patients with metastatic carcinoma (Senger *et al.*, 1988).

Most recently, Singh *et al.* (1990) identified a cytokine produced by activated T-lymphocytes, Eta-1 (early T-lymphocyte activation gene), which is now recognized to be OPN. While many other adhesion molecules have cytokine-like motifs, the classical cytokines/growth factors are not considered to be adhesion molecules. Similar to other lymphokines, studies on the activity of eta-1 secreted by activated T-lymphocytes indicate that, *in vitro*, eta-1 binds to macrophages, and *in vivo* administration of eta-1 in mice results in local infiltration of inflammatory cells, comprised mainly of macrophages (Singh *et al.*, 1990). To our knowledge, this is the first situation where a specific molecule can be considered both a cytokine and an attachment protein. Studies to date indicate that OPN interacts with the $\alpha_v\beta_3$ integrin receptor on fibroblasts, *i.e.*, antibodies to $\alpha_v\beta_3$ block OPN-mediated fibroblast attachment (Somerman *et al.*, 1990; Sauk *et al.*, 1991); however, a specific receptor for OPN on fibroblasts or lymphocytes has yet to be established. A 90-kDa fibroblast cell-surface glycoprotein has been identified, by OPN-CNBr Sepharose affinity chromatography, as a potential receptor for OPN, and appears to be distinct from $\alpha_v\beta_3$, β_1, and β_2 integrins (Somerman *et al.*, 1992). It will be interesting to determine the specific properties of this glycoprotein and to estabtish whether or not a receptor with similar properties is present on the cell surfaces of activated lymphocytes. Although the exact function of OPN is unknown, possibilities include a role for the recruiting of cells to a site of mineralization, a role in promotion of cell metastasis by adhesive interactions at secondary sites, and a role in protection against bacterial infection.

Adhesion Molecules and Implant Surfaces

The possible role of OPN in initiation of mineralization and its ability, unlike fibronectin, to promote both primary cell attachment and later stages of

spreading and adhesion prompted us to examine this persistent spreading in more detail (Somerman *et al.*, 1987; Sauk *et al.*, 1990a,b). The long-term plan is to develop synthetic peptides that can be used clinically for enhancement of attachment of cells at a specific site, *e.g.*, implant surfaces. For these initial studies, dishes coated with OPN, fibronectin, and collagen, or with synthetic peptides containing an RGD sequence, were evaluated for their ability to enhance attachment and the spreading of fibroblasts and for their ability to protect cells from heat shock. The rationale for consideration of a protective role for these molecules was two-fold: (1) *In vitro* exposure of cells to heat stress stimulates expression of heat-stress proteins (hsps). It is generally accepted that cells respond to heat and other environmental stresses, *e.g.*, cytokines and immune mediators, by synthesizing a small set of stress proteins and by inhibiting the post-translational synthesis of normal proteins (Craig and Jacobsen, 1985; Schlesinger, 1986). These proteins are thought to have protective roles in traumatic situations, such as would be experienced at an implant site initially. (2) Data from our laboratories as well as others' support the concept that a link exists between stress tolerance and adhesion proteins (Sauk *et al.*, 1990a,b, 1991). Polla *et al.* (1988) noted that 1,25 dihydroxyvitamin D_3 maintains the attachment of monocytes to tissue culture plates and biological matrices, *in vitro*, enhances the synthesis of hsps, and protects the cells from thermal injury. Other studies have shown that 1,25 dihydroxyvitamin D_3 enhances OPN synthesis by bone cells (Prince *et al.*, 1987), and that hsps stimulate synthesis of certain adhesion molecules, *e.g.* thrombospondin (Ketis *et al.*, 1988).

Fig. 2 summarizes the results of our studies (Sauk *et al.*, 1990a,b, 1991) indicating that cell spreading and attachment correlate with enhanced stress tolerance by fibroblasts. By 1.5 h, significant attachment of cells to all substrata was observed, although attachment to synthetic peptides was approximately 25% of that observed for collagen, fibronectin, or OPN-mediated cell attachment (data not shown; Sauk *et al.*, 1990a). Depicted in Fig. 2 is the percent of attached cells that were spread at 1.5 h and 24 h, and the survival of cells exposed to heat stress, 43°C for 3 h, at these same time points. Tolerance to stress is maintained by cells attached and spread long-term on substrata coated with type I collagen and OPN, but not in situations where cell-spreading is not maintained long-term, *e.g.*, fibronectin- and RGD-peptide-coated dishes. These results suggest that specific proteins or synthetic molecules may prove beneficial for enhancing cell attachment and spreading of cells to artificial devices and thus may enhance the predictability of implant-tissue integration. Equally important for implant success is recognition of early signs of potential failure and subsequent designing of clinical treatments for early intervention.

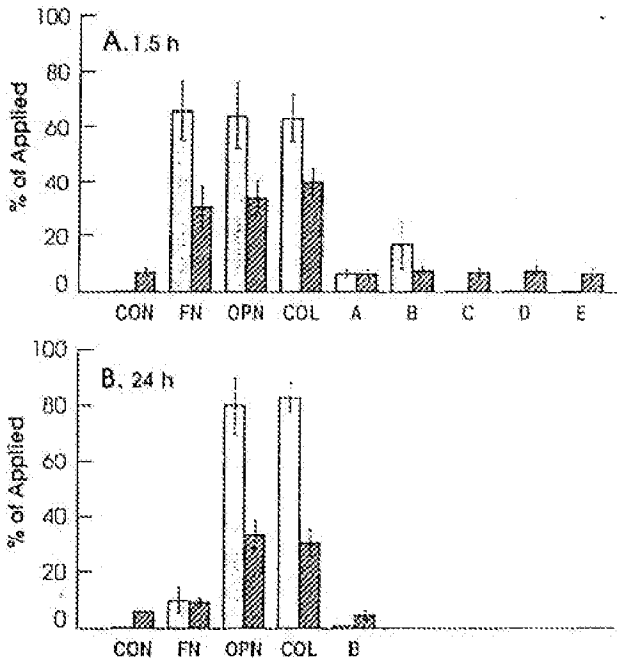

Figure 2. Spreading and survival of osteoligament cells on protein and peptide-coated substrata before and after exposure to heat stress. Open bars represent cell-spreading prior to heat stress, and hatched bars represent survival of cells after exposure to 43°C for three h. (A) 1.5 h. (B) 24 h. Abbreviations: CON = control, FN = fibronectin, OPN = osteopontin, COL = collagen. Synthetic peptides: A = fibronectin, VTGRGDSPA[C]; B = fibronectin, VTGRGDSPASSKPI[C]; C = osteopontin, FDGRGDSLAYGLRS[K]; D = vitronectin, QVTRGDVFTMPEDE[K]; E = bone sialoprotein, ERGDTYRAVEDE[K].

Peri-implantitis

In 1992, it is estimated that, in the United States' population alone, more than 300,000 dental implants will be placed, and this number is expected to increase even more in the coming years (Worthington, 1988). Most titanium implant systems report a 90% or greater success rate (Albrektsson and Sennerly, 1991). Some of the successful implants can have a less-than-complete degree of osseointegration, *i.e.*, buccal or lingual dehiscence. In addition, the number of failing implants will present the dental profession with a new challenge, the challenge of maintaining and restoring these fixtures to function (Worthington *et al.*, 1987; Becker *et al.*, 1990a). Implant failure appears to be the result of several factors: occlusal or parafunctional forces, premature loading, ill-directed stress (Van Steenberghe *et al.*, 1990; Zarb and Schmitt, 1990; Brånemark, 1985), and microbial invasion (Becker *et al.*, 1990b; Newman and Flemming, 1988; Apse, 1988; Brandes *et al.*, 1988; Holt *et al.*, 1986; Mombelli *et al.*, 1987;

Haanaes, 1990; Hickey *et al.*, 1991). Regardless of the cause, the end-result is the same, peri-implantitis. The clinical picture of peri-implantitis can include muscosal inflammation, increasing attachment loss, possible exposure of a portion of the fixture to the oral environment, radiographic evidence of bone loss, and/or the potential loss of the fixture over time.

Implant fixtures must be surgically placed under sterile conditions so that contamination of the fixture surface can be prevented. The surgery must be as precise and as atraumatic as possible, with use of instruments that create a congruent fit without a surface space between vital bone and the fixture (Eriksson, 1984; Carlsson *et al.*, 1988; Caudill and Meffert, 1991). Knox *et al.* (1991) demonstrated that if a space of 0.5 mm or more exists between the implant surface and the osseous wall, the level of osseointegration was significantly less than that found when the fixture was congruent with bone. Ideally, the fixture should be isolated from the oral environment, and loading of the implant should be delayed for three to six months.

Once an implant system with the prosthetic component is functional, and peri-implantitis occurs, two major problems make the likelihood of a new osseointegration unpredictable. First, the techniques for restoration of the fixture surface *in vivo* have not been developed, and, second, the techniques needed for restoration of new healing bone in close contact with the fixture have not been refined.

Presently, the only acceptable methods of sterilizing titanium are *in vitro* and include high heat, electropolish, sand-blasting, and acid-pickling. The highest percentage of bone-implant interface was observed on sand-blasted and acid-etched surfaces (large grit; HCL/H_2SO_4) (Buser *et al.*, 1991), with the titanium then stored under a vacuum until ready for use.

Decontamination of the fixture *in vivo* is presently being accomplished by means of conventional periodontal regenerative techniques. These include removal of the granulation tissue around the fixture, debridement of the fixture with an air-abrasive unit, and then treatment of the surface with either a citric acid for three min or with a tetracycline solution for five min. These techniques are acceptable for tooth surface preparation, but whether they are adequate for the titanium surface to be restored to its ideal properties needs further research.

Case reports have indicated the usefulness of conventional periodontal regeneration techniques in the treatment of failing implants. After fixture preparation, the osseous defects are usually grafted with demineralized cortical bone powder, demineralized freeze-dried bone and/or hydroxyapatite (40-60-mesh) (Gammage *et al.*, 1989; Lozada *et al.*, 1990). Although these techniques appear to be clinically successful, minimum follow-up and no histology are available to support these methods for treatment of peri-implantitis.

The concept of guided tissue regeneration (GTR) by means of a polytetrafluoroethylene (PTFE) membrane has been used with implants (Buser *et al.*, 1990; Zablotsky *et al.*, 1991). The PTFE was designed to enhance regeneration of the lost attachment apparatus of the teeth affected with periodontitis by exclusion of fibroblast and epithelial cells during the early stages of wound healing (Nyman *et al.*, 1982; Gottlow *et al.*, 1984). This principle has been expanded and applied in a series of experimental studies for the regeneration of bone tissue in different types of jaw bone defects, as well as around dental implants (Dahlin *et al.*, 1989; Seibert and Nyman, 1990; Becker *et al.*, 1990a). In association with dental implants, GTR (E-PTFE) has been developed and is considered for several treatments, including (1) ridge augmentation and subsequent placement of a new fixture, and (2) treatment of peri-implantitis defects in an already-osseointegrated fixture.

In animal studies, GTR has been successful in ridge augmentation and subsequent placement of a new fixture. Recently, studies have been designed for determination of the value of GTR membranes for the treatment of implantitis. Singh *et al.* (1992) have used a ligature/induced implantitis model to address such issues. Using conventional fixture decontamination and E-PTFE, they evaluated SEM-level histological specimens that demonstrated a significant gain in bone height (mean = 2.13 mm). There was, however, only a 36% zone of new osseointegration at the base of the defect, and the other 64% of the exposed fixture had a soft-tissue interface between the new bone and the fixture.

Clearly, additional research is needed in this area. As the number of implants placed increases, the need for understanding of the failure modalities is rapidly becoming compelling. To respond to this need, major research efforts should include the following: (1) exploration of individual predisposition to implant failure by identification of factors, both biological and mechanical, that promote the failure seen clinically; (2) development of methods for early recognition of potential factors which contribute to implant failure; (3) establishment of clinical procedures that would enhance the biocompatibility of host with implant materials before placement of the implants; and (4) once potential failure is recognized, development of predictable techniques for correction of the loss of osseointegration. For example, research is needed for the severe obstacles to successful osseointegration to be overcome in instances where the space between the implant and bone is 0.5 mm or greater. Our studies, as well as those of others, strongly suggest that the use of selective growth factors and/or adhesion molecules, alone or in combination, in

biodegradable delivery systems with GTR materials may be effective approaches to this new challenge.

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This concludes The Michigan Seminar.